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CARDIOVASCULAR RISK FACTORS AND PHARMACOGENETICS OF CLOZAPINE IN SCHIZOPHRENIA

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CARDIOVASCULAR RISK FACTORS AND PHARMACOGENETICS OF CLOZAPINE IN SCHIZOPHRENIA

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To Emmy, my partner in life and mother to our two beloved children Sixten and Folke. Thank you for standing by my side, for your patience, laughter, and love and for giving me hope in difficult times.

ABSTRACT

Background: Severe mental illness, including schizophrenia is associated with an increased risk of premature death by somatic conditions and in particular by cardiovascular events. Reducing cardiovascular risk factors in patients with psychotic disorder is urgently called for.

Aim: This thesis describes frequencies of cardiovascular risk factors among patients with psychosis. Clinical and genetic factors affecting plasma concentration of clozapine and their respective relation to increased fasting glucose and waist circumference are evaluated. In the last part of the thesis, levels of three biomarkers associated with cardiovascular risk are determined among patients with psychosis.

Methods: In two papers (Studies I and III), patients with psychosis recruited from outpatient clinics in Stockholm County Sweden were compared to non-psychotic control subjects from the population. Clinical and genetic factors related to plasma concentration of clozapine were explored in a sample of 113 patients on clozapine treatment (Study II). In one study (Study III), pharmacological treatment of diabetes was compared between 977 patients with psychosis and 3908 control subjects. In patients with psychosis and diabetes, factors associated with pharmacological treatment of diabetes were determined. In the last manuscript (study IV), levels of the biomarkers hsTnT, hsCRP and NT-BNP were determined in 300 patients with psychotic disorder.

Results: Psychosis was associated with higher fasting glucose levels, increased waist circumference and higher frequency of smoking compared to controls. Furthermore, 50 percent of patients with psychosis fulfilled the criteria for metabolic syndrome. Both prediabetes and diabetes were approximately three times more frequent among patients with psychosis as compared to controls. Of patients with psychosis and prediabetes 77 percent also fulfilled the criteria for metabolic syndrome. Genetic variants of *CYP1A2* and *MDR1*, and smoking were associated to lower plasma concentrations of clozapine. Levels of biomarkers, in previous studies associated with elevated cardiovascular risk, were elevated above reference values in patients with psychosis.

Conclusion: Reduced conversion from prediabetes to manifest diabetes is proposed as a treatment goal for patients with psychosis. Regular therapeutic drug monitoring is advised when clozapine is used. Longitudinal evaluation of biomarkers of cardiovascular risk would add valuable information to the clinician caring for patients with psychosis.

Keywords: Cardiovascular risk, psychosis, metabolic syndrome, diabetes, prediabetes, waist circumference, smoking, healthy individuals, clozapine, plasma concentration, genetics, single nucleotide polymorphisms (SNPs), CYP1A2, MDR1, high-sensitive troponin T (hsTnT), high-sensitive C-reactive protein (hsCRP), N-terminal pro-B-type natriuretic peptide (NT-BNP)

LIST OF SCIENTIFIC PAPERS

This thesis is based on three published articles and one manuscript. The individual studies will be referred to by their Roman numerals I-IV in the text.

- I. Osby U, Olsson E, Edman G, Hilding A, Eriksson SV, Ostenson CG. Psychotic disorder is an independent risk factor for increased fasting glucose and waist circumference. *Nord J Psychiatry* 2014;68:215-8. doi: 10.3109/08039488.2013.797022
- II. Olsson E, Edman G, Bertilsson L, et al. Genetic and Clinical Factors Affecting Plasma Clozapine Concentration. *The primary care companion for CNS disorders* 2015;17(1) doi: 10.4088/PCC.14m01704
- III. Olsson E, Westman J, Hukic DS, et al. Diabetes and glucose disturbances in patients with psychosis in Sweden. *BMJ Open Diabetes Research & Care* 2015;3(1) doi:10.1136/bmjopen-2015-007628
- IV. Olsson E, Forsberg S, Sudic Hukic D, Westman J, Schalling M, Eriksson SV, Edman G, Ösby U. Cardiac biomarkers in patients with psychotic disorder in Sweden.

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
SMR	Standardized mortality rate
BMI	Body mass index
IDF	International Diabetes Federation
DM	Diabetes mellitus
DRD2	Dopamine receptor D2
FGA	First generation antipsychotic
SGA	Second generation antipsychotic
QTc	Corrected QT interval
SMRP	Swedish study of metabolic risk in psychosis
CYP	Cytochrome P450
CYP1A2	Cytochrome P450 1A2
UGT1A4	Uridine diphosphate glucuronosyltransferase 1A4
MDR1	Multidrug resistance protein 1
PAH	Polycyclic aromatic hydrocarbon
SDPP	Stockholm diabetes prevention program
FPG	Fasting plasma glucose
FHD	Family history of diabetes
SNP	Single nucleotide polymorphism
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
NT-BNP	N-terminal pro-B-type natriuretic peptide
HsCRP	High-sensitive C-reactive protein
HsTnT	High-sensitive troponin T
MANCOVA	Multivariate analysis of variance and covariance
HDL	High-density lipoprotein
TG	Triglycerides
LDL	Low-density lipoprotein
HGO	Hepatic glucose output
IPS	Induced pluripotent stem cells

CVD

Cardiovascular disease

1 INTRODUCTION

1.1 TIMELINE

My work in the psychiatric field started in 2007 when I became a resident physician at the psychiatric clinic at Danderyd University Hospital. Urban Ösby who later became my main supervisor was working at the clinic and introduced me to psychiatric research. Early on I found patients with psychotic symptoms to be engaging. It is difficult for me to understand how someone is not intrigued by these diseases. The core symptoms of psychosis, hallucinations and delusions ruthlessly show how important the mind is. Urban Ösby with his burning enthusiasm for this patient group inspired me enormously to start my research. During the first three years we were working on a project focusing on genetic risk factors for suicide among patients with schizophrenia. 480 patients with schizophrenia who had died from suicide were identified through the Patient Register and the Cause of Death Register of the Swedish National Board of Health and Welfare. The time period was 1974-2006 and four major forensic regions in Sweden: Stockholm, Uppsala, Linköping and Lund were included. We started collecting biological material from the autopsies of the cases. We also identified patients with schizophrenia from the same time period who had died by unnatural causes but where the cause of death had not been classified as suicide. Since there are no national guidelines or decision-trees in forensic medicine to standardize the diagnostic procedure for determining if the cause of death is suicide or not, we formulated an algorithm and evaluated the pathology records of each case. The forensic or pathology records encompass somatic and psychiatric medical information, police reports, autopsy protocols, as well as results from chemical, toxicological and microscopic examinations. We slowly also started experiencing problems with the extraction of deoxyribonucleic acid (DNA) from the paraffin embedded tissue samples. Filled with enthusiasm we had not anticipated this. Central to the problem is that DNA gets fragmented during the purification process. To small fragments means that genes, which are large fragments of DNA, are very difficult to amplify and analyze. Although this project now finally seems to pay off, analyzing RNA and gene expression in contrast to DNA, we back then had to change course for my PhD project. It is well known also to the public that patients with severe mental illness have higher mortality in suicide compared to the general population. Less appreciated, also among physicians and psychiatrists, is the fact that the overall difference in mortality comparing patients with psychosis and the general population is to a large extent caused by cardiovascular disease, other somatic conditions and suicide. Patients with psychosis die from ordinary causes but at younger age compared to the population. Why do these patients have a higher risk for these conditions? An important way to improve the prognosis of schizophrenia would be to study why these patients have increased risk of cardiovascular disease, and to use the answer as guidance for counteraction. I decided to have this as my PhD research field. As this thesis goes to print, the United Nations have recently adopted developmental goals. These goals include commitment to reduce by one third premature mortality from non-communicable diseases for all at all ages by 2030.

1.2 SCHIZOPHRENIA AND RELATED DISORDERS

Severe mental illness is often characterized by a diagnosis of non-organic psychosis, prolonged illness and disability (Schinnar, Rothbard, Kanter, & Jung, 1990). Schizophrenia, one of the most common severe mental illnesses, has an estimated point prevalence of about four per 1000 (Saha, Chant, Welham, & McGrath, 2005). The condition is considered to be a major contributor of the global burden of disease (Whiteford et al., 2013). In Sweden the psychiatric outpatient clinics are divided in two different types, psychosis outpatient clinics and general psychiatric outpatient departments. Somatic health problems are treated in separate general practice outpatient clinics. This division and sub-specialization, generally occurring in the health care system, might be problematic for patients with schizophrenia, possibly creating a barrier to somatic health care. As of today, there seems to be no evidence that distance between disciplines will decrease over time.

Schizophrenia, as well as other severe mental illnesses, are well known to be associated with elevated suicide rates, with five percent dying of suicide (Palmer, Pankratz, & Bostwick, 2005). Less widely appreciated is the fact that schizophrenia is associated with a clearly increased risk of premature death by many other somatic conditions (Brown, 1997). As a result, the risk of cardiovascular mortality in schizophrenia is doubled compared to the general population (Laursen et al., 2013; Osby, Correia, Brandt, Ekblom, & Sparen, 2000). Moreover, standardized mortality rates (SMRs) for schizophrenia seem to be worsening over time (Saha, Chant, & McGrath, 2007).

A spectrum approach to mental diseases means that linked conditions or symptoms are grouped together. Schizophrenia with this approach forms psychosis spectrum disorders or schizophrenia spectrum disorders. In this thesis, where psychosis patients from outpatient clinics were studied, we included all patients in regular treatment. Previous findings strongly suggest that biological mechanisms of mental disorders are more likely to be found with a spectrum approach as opposed to a strict categorical approach.

Physical health monitoring typically occurring in primary care settings, has been suggested to be performed by mental health care providers to detect and intervene risk factors for somatic illness (Marder et al., 2004). These recommendations include monitoring of body mass index (BMI), plasma glucose level, lipid profiles, side effects of medication and specific recommendations for cardiac monitoring (Marder et al., 2004). A similar approach with regular monitoring has been included in Swedish clinical guidelines concerning schizophrenia and other psychotic disorders since 2009 (Gothefors et al., 2010).

1.3 CARDIOVASCULAR RISK FACTORS

There are several risk factors for cardiovascular disease. Diabetes mellitus, elevated lipid levels, and hypertension, increased waist circumference and smoking are the most important. Severe mental illness has previously been shown to be associated with increased prevalence of metabolic changes (Goff et al., 2005; Mackin, Bishop, Watkinson, Gallagher, & Ferrier, 2007). The mechanisms behind these changes are probably complex and could be explained

by shared genetics, adverse effects of antipsychotic treatment or lifestyle factors (Saha et al., 2007).

1.3.1 Metabolic syndrome

The metabolic syndrome is a set of important cardiovascular risk factors. In the studies for the present thesis we have used the operational criteria for metabolic syndrome defined by the International Diabetes Federation (IDF). In this definition increased waist circumference (ethnicity-specific values: ≥ 94 cm in male and ≥ 80 cm in female European Whites) is a main criteria that has to be fulfilled for the condition to be present. Any two of the following four factors: elevated triglycerides (≥ 1.7 mmol/L), reduced HDL cholesterol (< 1.03 mmol/L in males and < 1.29 mmol/L in females), elevated blood pressure (systolic ≥ 130 mm Hg, diastolic ≥ 85 mm Hg, or antihypertensive treatment), and elevated fasting plasma glucose (≥ 5.6 mmol/L or antidiabetic drug treatment) must also be present (Alberti, Zimmet, & Shaw, 2006).

1.3.2 Smoking

Smoking is a risk factor associated with adverse health outcomes e.g. myocardial infarction, cerebrovascular incidents, hypertension and neoplasms. The prevalence of smoking in patients with schizophrenia varies between 40 and 60 percent in different studies with different samples (Mackin et al., 2007; Osborn, Nazareth, & King, 2006). Generally, hospital based samples have higher prevalence of smoking. The prevalence of smoking in the current material was 41 percent.

Smoking is often regarded as a self-medication to improve cognitive deficits, either attributable to the illness or side effects of antipsychotic medication. Several studies have shown that measures of attention are improved by smoking (Ahlers et al., 2014; Segarra et al., 2011). Interestingly a recent study has also suggested an association between smoking and increased risk of developing psychosis (Gurillo, Jauhar, Murray, & MacCabe, 2015). Despite the notoriously high prevalence of smoking among patients with psychotic disorders and the established association between smoking and adverse health outcomes, smoking cessation studies are scarce.

1.3.3 Diabetes and glucose disturbances

In the following studies of the thesis, the diagnostic criteria of the WHO and IDF were used to define diabetes and prediabetes. According to these criteria, diabetes mellitus (DM) is defined as fasting glucose ≥ 7.0 mmol/L and prediabetes as fasting glucose, 6.1–6.9 mmol/L (Alberti & Zimmet, 1998). The progression rates from prediabetes to type 2 diabetes differ by definition of prediabetes. Previous epidemiological studies suggest that approximately 25% of patients with prediabetes progress to type 2 diabetes in 5 years, 50% remain with prediabetes and 25% revert to normal (Larsson, Lindgarde, Berglund, & Ahren, 2000). Higher progression rates, especially among more obese subjects, averaging 10-12% per year, have been suggested in other studies (Knowler et al., 2002; Tuomilehto et al., 2001). Lifestyle

interventions and oral antidiabetic agents have been reported to reduce the progression from prediabetes to manifest type 2 diabetes. The drug metformin has been demonstrated to reduce incident type 2 diabetes by 31% compared to control subjects who received placebo (Knowler et al., 2002). Other pharmacological agents than metformin have also been studied but metformin remains the first hand choice due to its safety data, efficacy, and cost profile. Life style interventions have often been shown to be more effective than pharmacological treatment in reducing the progression. However, the positive effects of life style interventions appear to be difficult to maintain over the long term. Partly due to the risk of side effects, pharmacological interventions have been suggested primarily for patients with prediabetes at particular risk for developing diabetes.

1.3.4 Overweight, BMI and waist circumference

Obesity is the most potent acquired risk factor for developing type 2 diabetes. The age-adjusted relative risk of developing type 2 diabetes is ~10-fold higher for men with a BMI of 30 kg/m² relative to men with a BMI < 23 kg/m² and the risk is even higher for women, where a BMI of 30 kg/m² is associated with a ~30-fold higher risk (Chan, Rimm, Colditz, Stampfer, & Willett, 1994; Colditz, Willett, Rotnitzky, & Manson, 1995).

Especially intra-abdominal obesity is associated with cardiovascular disease (CVD), dyslipidemia, type 2 diabetes and insulin resistance (Despres, Lemieux, & Prud'homme, 2001; Eckel, Alberti, Grundy, & Zimmet, 2010; Emery, Schmid, Kahn, & Filozof, 1993). There are essentially two ways of measuring intra-abdominal obesity: waist circumference and waist-to-hip ratio. The two measurements both seem to be equal in predicting CVD risk (de Koning, Merchant, Pogue, & Anand, 2007). Since waist-to-hip ratio involves two measurements and waist circumference only one, the latter may be preferred by the clinician.

1.4 ANTIPSYCHOTIC DRUGS

Antipsychotic drugs have high affinity for the dopamine receptor D2 (DRD2) and the antipsychotic effect is thought to be a result of blocking of this receptor. Antipsychotic drugs are commonly divided into two groups, first-generation antipsychotics (FGAs) and second-generation antipsychotics (SGAs). This division is based on when the drugs were developed, with exception of clozapine, an SGA developed during the 1950s.

SGAs are associated with fewer extrapyramidal symptoms and have a similar efficacy compared to FGAs. SGAs also have a reduced risk of discontinuation and relapse compared to FGAs. However, SGAs and especially olanzapine and clozapine are associated with an increased risk of weight gain and metabolic abnormalities (Hert, Detraux, van Winkel, Yu, & Correll, 2012). Lack of the drug to alleviate symptoms associated with psychosis and side effects, such as weight gain, extrapyramidal symptoms or sedation are often primary reasons for discontinuation.

Antipsychotic drugs also to a varying degree seem to have direct cardiac effects. Both FGAs and SGAs have been reported to be associated to prolonged corrected QT interval (QTc)

(Elbe & Savage, 2010; Strom et al., 2011). Furthermore, antipsychotic drugs have also been associated with myocarditis and cardiomyopathy. This association seems to be most pronounced for clozapine (Coulter, Bate, Meyboom, Lindquist, & Edwards, 2001). Due to the potential side effects of antipsychotic drugs and the markedly increased risk of cardiovascular morbidity and mortality among psychosis patients, close monitoring of cardiovascular risk factors have been recommended. Regular assessment of fasting glucose, blood pressure, waist circumference and lipids have been suggested as important measures (Hert et al., 2012; Khasawneh & Shankar, 2014). Electrocardiography before initiation of antipsychotic treatment (Hert et al., 2012) and in the case of clozapine treatment, a low threshold to perform echocardiography have also been advocated (Alawami, Wasywich, Cicovic, & Kenedi, 2014).

1.4.1 Clozapine

Clozapine is an SGA with structural resemblance to olanzapine. It was first synthesized in 1958 (Crilly, 2007). Since its introduction, withdrawal and re-introduction on the market, clozapine stands out compared to other antipsychotic drugs with regard to both efficacy and side effects. It is considered especially effective in treatment-resistant schizophrenia (J. Kane, Honigfeld, Singer, & Meltzer, 1988; J. M. Kane, 1996). Furthermore, clozapine is the only antipsychotic drug with a significant association to reduced suicidality (Hennen & Baldessarini, 2005). Considering side effects, clozapine seems to have a high propensity to produce weight gain and metabolic disturbances. Agranulocytosis as a potential side effect is well recognized and regular monitoring of white blood cells is mandatory in many countries including Sweden. Recently, a less rigorous long time white blood cell monitoring has been proposed in Sweden (Olsson, Lennestal, & Hagg, 2015). Maybe less appreciated is clozapine's ability to cause electroencephalographic changes and seizures (Leucht et al., 2013).

An overview of clozapine treatment in Sweden during 2012 is presented in Table 1. During 2012 approximately 18 percent of patients with a diagnosis of schizophrenia or schizoaffective disorder with antipsychotic treatment were treated with clozapine. Since the Drug Prescription Register of the Swedish National Board of Health and Welfare was not in full use until the end of 2006, a washout period from 2007-2011 was used for incident cases of clozapine during 2012. The time period 2007-2012 was used to calculate duration of treatment for all patients with clozapine and for patients discontinuing treatment. The time to discontinuation of treatment has previously in several naturalistic studies been shown to be longer for atypical than typical antipsychotics. Furthermore, within the group of atypical antipsychotics clozapine and olanzapine seem to contribute most to longer duration to treatment discontinuation (Ascher-Svanum et al., 2006).

Table 1. Antipsychotic treatment and duration of clozapine treatment in Sweden during 2012 according to data from the Swedish Drug Prescription Register, National Board of Health and Welfare*

Schizophrenia or schizoaffective disorder	18 114	Duration of treatment (years)
Antipsychotic treatment	16 964	ND
Clozapine treatment	3 003 (18)	4.8 (5.8)
Discontinued clozapine treatment	180 (6)	2.9 (2.9)
Started clozapine treatment	551 (18)	NA

*Data reported as number, number (%) or mean (median)

NA, not applicable; ND, not determined

1.4.2 Drug-metabolizing enzymes and transport proteins

Many different enzymes are involved in drug metabolism, including the hepatic cytochrome (CYP) P450s which are important for the metabolism of many drugs including antipsychotics (Sheweita, 2000). Concerning clozapine, the isoenzyme CYP1A2 is thought to be primarily responsible for the metabolism (Bersani et al., 2011). Other enzymes such as uridine diphosphate glucuronosyltransferase 1A4 (UGT1A4) have also been suggested to affect inter individual variability (Erickson-Ridout, Sun, & Lazarus, 2012). Multidrug resistance protein 1 (MDR1), a transport protein expressed in the liver, kidneys and the blood-brain barrier may also affect plasma concentration and consequently the pharmacological effect of the compound (Bersani et al., 2011; Consoli et al., 2009).

1.4.3 Investigated genes regulating metabolism of clozapine

The genes investigated in this thesis encode the enzymes and the transport protein involved or suggested to be involved in the metabolism of clozapine. A functional variant of a gene affecting metabolism should be reflected in a different concentration of the drug metabolized by the enzyme. The specific variants of the genes and their respective suggested effect on function of the protein are shown in Table 2.

The *CYP1A2* gene is located on chromosome 15q24.1 and encodes a protein which catalyzes many reactions involved in drug metabolism. The enzyme mediates the rate-limiting step in the metabolism of different drugs including clozapine, olanzapine and caffeine (Faber, Jetter, & Fuhr, 2005). *CYP1A2* is expressed mainly in the liver and is induced by polycyclic aromatic hydrocarbons (PAHs) found in cigarette smoke (Bersani et al., 2011).

The *MDR1* gene is located on chromosome 7q21.1 and encodes a P-glycoprotein which functions as efflux pump from the inside of the cell to the outside (Brinkmann, 2002).

The *UGT1A4* gene is located on chromosome 2q37 and encodes an enzyme regulating the transformation of drugs into water soluble metabolites. In the case of clozapine, UGT1A4 has been suggested to affect inter individual variability of plasma concentration (Erickson-Ridout et al., 2012).

Table 2. Genetic variants studied and corresponding functional effect

Gene and Single Nucleotide Polymorphism	Functional effect
<i>CYP1A2</i>	
CYP1A2*1D -2467delT rs35694136	Unknown
CYP1A2*K -729C>T rs12720461	Activity ↓
CYP1A2*F -163C>A rs762551	Activity ↑
<i>MDR1</i>	
-3435C>T rs1045642	(Expression ↓)
-2677G>T rs2032582	Unknown
<i>UGT1A4</i>	
L48V -142T>G rs2011425	Activity ↓

2 AIMS

The main objectives of this thesis were (1) to assess cardiovascular risk factors and pharmacological treatment of diabetes, and (2) to assess the clinical relevance of genetic variants on clozapine plasma concentration in patients with psychotic disorder.

The specific aims related to each paper were:

- To assess differences in waist circumference, fasting glucose and tobacco use in patients with psychotic disorder compared to population controls from the same geographical area (Study I).
- To assess the effect of age, gender, smoking status, weight and genetic variants of *CYP1A2*, *MDR1* and *UGT1A4* on plasma levels of clozapine (Study II).
- To study relation between plasma concentrations of clozapine and waist circumference and fasting glucose levels (Study II).
- To assess prevalence of diabetes, prediabetes and antidiabetic treatment in patients with psychotic disorder compared to population controls (Study III).
- To study what factors are associated to pharmacological antidiabetic treatment when diagnosed with diabetes and a psychotic disorder (Study III).
- To determine levels of three biomarkers associated with cardiovascular risk in 300 psychosis patients (Study IV).

3 METHODS

3.1 STUDY POPULATION SWEDISH STUDY OF METABOLIC RISKS IN PSYCHOSIS (SMRP)

The study sample of this thesis emanates from a sample of psychosis patients recruited during the years 2005-2010 in the Swedish Study of Metabolic Risks in Psychosis (SMRP). In Sweden, special psychiatric outpatient clinics have treatment responsibility for all patients with chronic psychotic disorders. The patients were recruited consecutively from psychosis outpatient clinics mainly in Stockholm County, Sweden as a part of an assessment of general health. Of the total number of patients, 75 percent were recruited in Stockholm County.

Psychiatric diagnoses were confirmed by the clinicians according to the DSM-IV (American Psychiatric Association., 2000). Data was collected with a psychiatric questionnaire containing information on diagnosis, global assessment of functioning (GAF) (Aas, 2011) and clinical global impression (CGI) (Busner & Targum, 2007), duration of illness, duration of treatment, duration of hospitalization during the last year. All present medication, including dosages, were confirmed in case notes and recorded.

Patients with psychosis were assessed with a questionnaire about somatic health. This questionnaire included perceived degree of health, questions about diabetes, hypertension, current tobacco use, and previous diagnosis of myocardial infarction. It also included questions covering alcohol use, chest pain during the last year, dyspnea and diabetes among first-degree relatives. Waist circumference, blood pressure, length and body weight were measured. Cases were given written instruction to be fasting overnight before venous blood sampling.

3.2 CONTROL SUBJECTS STUDY I AND III

The control subjects in study I and III were participants in a follow-up study of a population based survey, the Stockholm Diabetes Prevention Program (SDPP). The follow-up study was performed during 2002-2006, eight to ten years after baseline. The sample in the follow-up is described in detail in previous studies (Eriksson et al., 2008). Since one of the main aims of the follow-up study was to investigate the effect of family history of diabetes on the risk of glucose disturbances, the whole sample was selected from all responders and enriched for family history of diabetes. The enrichment was made so that half had a family history of diabetes. Concerning diabetes, patients who already had a diagnosis or were diagnosed during the health examination at baseline, were excluded from the follow-up. Hence, diabetes cases among controls in the present thesis, are only subjects who developed diabetes between baseline and follow-up.

All present medication and dosages were recorded. Controls were asked about family history of diabetes, defined as at least one first-degree relative with known diabetes. Somatic health was assessed with a questionnaire, covering diabetes, hypertension, and tobacco use. Body weight, height, waist circumference and blood pressure were measured.

Fasting plasma glucose (FPG) was measured in all controls. In addition, controls without a diagnosed diabetes went through an oral glucose tolerance test.

3.3 METHOD FOR EACH PAPER

3.3.1 Study I

In the first study of the thesis 731 patients with psychotic disorder, recruited consecutively from outpatient clinics in Stockholm were compared to 5580 population controls with respect to cardiovascular risk factors. The cardiovascular risk factors of main interest were waist circumference, body mass index and fasting glucose.

3.3.1.1 Methodological considerations

The study design enabled us to compare frequencies of cardiovascular risk factors between a large outpatient sample and a population based sample of controls. Hence, the results could be generalizable to the population of psychosis patients. There were significant differences between cases and controls in age, with controls being older and in family history of diabetes (FHD), with higher frequency of FHD among controls. In the statistical comparisons these factors were controlled for, however their presence warrants some caution when interpreting results. The exclusion of diabetes cases among controls at baseline could bias the results, since diabetes cases among controls in this study were only those who developed diabetes between baseline and follow up. In other words patients had a longer period of time, during which they could develop diabetes. Antipsychotic treatment has been suggested as an important cause of metabolic disturbances. Unfortunately, since a vast majority of patients were on current treatment and no patient was likely drug naïve it was not possible to analyze the independent effect of antipsychotic medication. We had no socioeconomic data, data on dietary habits or exercise and therefore the effects of these variables were not possible to analyze.

3.3.2 Study II

Of the 731 patients in the first study, 113 (15%) were prescribed clozapine, including 74 patients having clozapine as monotherapy. Blood samples for genetic analyses were available in 95 of the 113 patients and in 98 patients plasma concentrations of clozapine and the major metabolite norclozapine were measured. Patients were given written instructions to be fasting overnight and not to take their morning dose of clozapine, if prescribed twice daily, before venous blood sampling. Information on daily dose clozapine, concomitant medication, main psychiatric diagnose, fasting glucose, waist circumference, body weight, sex and smoking habits were recorded. Concurrent drugs possibly affecting CYP1A2 activity were identified.

3.3.2.1 Methodological considerations

The major strength with the study is that it is a clinical sample of patients who are being treated with clozapine. However this strength of the study also implies weaknesses. Most patients were on monotherapy with clozapine but 32 percent were taking a combination of

clozapine and some other antipsychotic drug. This could bias the results. Furthermore, one important weakness was that we had no estimate of the effect of clozapine. It is plausible that patients on very low doses of clozapine did not have clozapine as main antipsychotic pharmacotherapy and that low concentrations are effective in improving symptoms such as insomnia. Concerning compliance to the prescribed treatment, we had no other measurement than the plasma concentration. It is however noteworthy that all patients had concentrations above detection level. Blood samples for measurement of plasma concentration were all taken in the morning. Unfortunately the exact time for sampling was not standardized. To compensate for this a time factor was introduced based on the average half-life of clozapine. This average half-life however, is also an approximation.

3.3.2.2 Genotyping

Three different techniques were used to genotype the single nucleotide polymorphisms (SNPs) of interest. The different techniques used were the TaqMan method, pyrosequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Reaction conditions, sequences of interest, primers and rs numbers and the restriction enzyme are described in detail in the supplementary material to study II.

3.3.3 Study III

In this study 977 patients with psychotic disorder recruited in SMRP were compared to 3908 population controls. The controls were randomly selected, matched on frequency of FHD among patients with psychosis (24%). We assessed differences between the groups in fasting plasma glucose levels, frequencies of diabetes, prediabetes, pharmacological antidiabetic treatment, and smoking habits. Differences in waist circumference, blood pressure and body mass index were also evaluated. In patients with a psychotic disorder and a diabetes diagnosis, factors associated with antidiabetic pharmacological treatment were studied.

3.3.3.1 Methodological considerations

As mentioned for controls in study I, the present controls included only incident diabetes cases diagnosed between baseline and follow-up. In this regard, cases and controls were not comparable in respect to occurrence of diabetes. The control subjects were nine years older than the patients. Since age could affect the frequency of diabetes, the comparisons were statistically controlled for differences in age. Controls did not go through a psychiatric assessment and therefore patients with psychosis might be present among controls biasing results. However, in retrospect the medication of controls was evaluated to detect antipsychotic drugs. In this evaluation, four controls had recorded use of antipsychotic drugs. For patients with psychosis, we had data on frequencies of the metabolic syndrome, and controls without a diagnosed diabetes went through an oral glucose test but not vice versa. Thus, comparison of the metabolic syndrome between cases and controls was not possible and the diagnosing of diabetes was more thorough among controls. It is well known that there is a considerable intrapersonal variance of fasting glucose over time. Hence it would have been better to use glycated hemoglobin, much more stable over time, as an assessment of

antidiabetic pharmacological treatment. Also, the use of one fasting glucose value when diagnosing diabetes could bias the results if compliance to fasting instructions before blood sampling differs between patients with psychosis and controls.

3.3.4 Study IV

Levels of three biomarkers, in previous work associated with increased cardiovascular risk, were studied in 300 patients with psychotic disorder, recruited in SMRP. The three biomarkers of interest were: N-Terminal Pro-B-Type Natriuretic Peptide (NT-BNP), high-sensitive troponin T (hsTnT) and high-sensitive C-reactive protein (hsCRP). Data on serum lipid profiles, plasma creatinine and fasting plasma glucose were also available. Somatic health assessment was performed with a questionnaire about diabetes, hypertension, and previous diagnosis of myocardial infarction and current use of tobacco. Waist circumference, blood pressure, length and body weight were measured. In this cross-sectional study, our aim was to evaluate levels of biomarkers and assess potential associations between biomarkers and traditional clinical cardiovascular risk factors.

3.3.4.1 Methodological considerations

To study potential advantage of biomarkers of increased cardiovascular risk over traditional clinical risk factors and scoring systems, one would have to perform a longitudinal study containing cardiovascular events. This is of course a very interesting question for the clinician. Unfortunately, the current study design did not enable us to study this question. Hence, the study-design could be considered as a major weakness. On the other hand, one could argue that it is wise to test if patients with psychosis have pathological levels of biomarkers before starting a longitudinal study.

3.4 STATISTICAL ANALYSIS

3.4.1 Study I

Standard descriptive statistics were used to summarize all variables. Multivariate analysis of variance and covariance (MANCOVA) was used to study differences between patients and controls in continuous variables. In this analysis age and gender were entered as covariates because of the significant difference between cases and control subjects in these variables. Differences in categorical variables between cases and controls were evaluated with the χ^2 test. Associations between increased fasting glucose and increased waist circumference as dependent variables, and gender, group (control subject/patient with psychosis) and current tobacco use were tested with logistic regression.

3.4.2 Study II

Standard descriptive statistics were used to summarize all variables. Plasma concentration of clozapine corrected for daily dose, ratio of concentration to dose, was positively skewed. Ratios of plasma concentration to dose clozapine were therefore analyzed with the Mann-Whitney test for differences between patients with different sex, age (<44 y, \geq 44 y), smoking status, body weight (<90 kg, \geq 90 kg) and genetic variants. The genetic variants were coded as dichotomous variables under a dominant or recessive model. The combined effect of genotype and clinical variables on plasma concentration to dose ratio clozapine was analyzed with linear regression. Logistic regression models were used to study potential associations between genetic variants, clinical variables and plasma concentration of clozapine with increased waist circumference and increased fasting glucose as dependents variable in each model respectively.

3.4.3 Study III

Standard descriptive statistics were used to summarize all variables. Analysis of variance and covariance (ANCOVA) with age and sex as covariates was used to test differences in continuous variables between patients with psychosis and controls for significance. Differences in categorical variables such as tobacco use and sex between patients and controls were analyzed with the χ^2 test. The categorical variables antidiabetic pharmacological treatment (no/yes) and achievement of FPG <7 mmol/L (no/yes) were analyzed in logistic regression models with age, sex and group (patients with psychosis or controls) as independent variables. Among patients with psychosis and diabetes, associations between antidiabetic pharmacological treatment and clinical variables were evaluated. In this evaluation logistic regression was used to study the relation between antidiabetic pharmacological treatment as dependent variable and smoking status (ref = non-smoker), fasting plasma glucose (continuous), the metabolic syndrome (ref = no), waist circumference (continuous), Global Assessment of Functioning scale (continuous), Clinical Global Impression scale (continuous), antihypertensive treatment (ref = no), lipid-lowering treatment (ref = no), duration of antipsychotic medication (months, continuous variable), and number of months in hospital during the previous year (continuous variable) as independent variables.

We also performed two separate regression analyses with waist circumference and fasting glucose as dependent variables with clinical variables including duration of antipsychotic treatment (continuous), age (continuous), main psychiatric diagnosis (categorical) and sex (ref = man) as independent variables.

3.4.4 Study IV

Standard descriptive statistics were used to summarize all variables. The distributions of the biomarkers were positively skewed and were summarized with mean, median and standard deviation. To study univariate relations, non-parametric correlation coefficients (Kendall's tau-b) were calculated between biomarkers and the clinical variables. Differences in levels of biomarkers and categorical variables with two categories were evaluated with the Mann-Whitney test. Differences in levels of biomarkers and categorical variables with more than two categories were evaluated with the Kruskal-Wallis test. To study relations between clinical cardiovascular risk factors and biomarkers, multiple regression models were used. In these models biomarkers were dependent variables and fasting glucose, waist circumference, high-density lipoprotein (HDL), triglycerides (TG), low-density lipoprotein (LDL), systolic blood pressure, diastolic blood pressure, smoking, age, sex and creatinine level independent variables.

3.5 ETHICS APPROVAL

The studies in the present thesis have ethics approval and were conducted in accordance with the Declaration of Helsinki. Ethics approval was obtained separately for cases from the Stockholm Regional Ethics Committee (dnr 2004/4:6, 2006/249-32) and controls from the Ethics Committee of the Karolinska University Hospital (dnr 91-164, 95:298). All participants gave informed consent.

4 SUMMARY OF RESULTS

4.1 PAPER I

Mean waist circumference in patients compared to controls was for males 106 and 94 cm, respectively, and for females 97 and 85cm, respectively ($P < 0.001$). Mean fasting plasma glucose in patients compared to controls was for males 5.8 and 5.2 mmol/L, respectively, and for females 5.6 and 4.8 mmol/L, respectively ($P < 0.001$). Since there were significant differences in age and family history of diabetes in cases and control subjects, and these variables affect the outcome of interest, these variables were controlled for in the comparative analyses. Fifty-three percent of the patients were current smokers and/or snuff users compared to 25 percent of the control subjects ($\chi^2 = 2\ 240.02$, $P < 0.001$). Fifty percent of patients fulfilled the criteria for metabolic syndrome as defined by the International Diabetes Federation.

Risk factors for increased waist circumference and increased fasting glucose were assessed in two logistic regression models. Group (control vs. patient), gender, age, tobacco use, family history of diabetes, fasting glucose, fasting insulin and blood pressure were entered as independent variables with waist circumference as dependent variable. In the second model with fasting glucose as dependent variable the same variables were entered as independent variables with the addition of waist circumference. The first regression model showed that all variables entered were significantly associated to increased waist circumference. Controlling for other risk factors entered in the model, increased waist circumference was almost four times (95% CI 3.09 – 5.15) as common among patients with psychosis compared to controls. There was no excess risk for increased waist circumference comparing patients with schizophrenia and schizoaffective disorder to patients with other diagnoses. In the second regression model all variables entered showed significant associations to increased fasting glucose. Increased fasting insulin was associated with the highest risk for increased fasting glucose (OR = 3.19; 95% CI 2.74 – 3.73). Psychosis was also associated with an increased risk for higher fasting glucose (OR = 2.41; 95% CI 1.84 – 3.14). For the variable gender, the relation to increased fasting glucose was reversed compared to the risk of increased waist circumference, with higher risk for men (OR = 0.45; 95% CI 0.38 – 0.52). Results from the two regression analyses are shown in Table 3 and 4.

Table 3. Odds ratios (OR) after a logistic regression analysis (stepwise forward) for eight independent variables related to increased *waist circumference*.

Independent variable*	b	SE	(OR)†	95% CI	
				Lower	Upper
Group	1.38	0.130	3.99	3.09	5.15
Fasting insulin	1.34	0.070	3.81	3.32	4.37
Gender	1.11	0.069	3.04	2.65	3.48
Blood pressure	0.82	0.073	2.27	1.97	2.62
Fasting glucose	0.68	0.083	1.97	1.68	2.32
FHDf	0.19	0.063	1.21	1.07	1.37
Age	-0.18	0.065	0.84	0.74	0.95
Tobacco use	0.17	0.071	1.19	1.03	1.36
Constant	-4.94	0.203			

CI = Confidence Interval

*Group (control vs. patient); gender (male vs. female); age (≤ 55 vs. >55 yrs); tobacco use (smoking or snuffing – no vs. yes); first-degree relative with diabetes (FHD – no vs. yes); fasting glucose (≥ 5.6 mmol/L – no vs. yes); fasting insulin (<114 pmol/L vs. ≥ 114 pmol/L); and blood pressure (systolic ≥ 130 mmHg or diastolic ≥ 85 ; and mmHg or antihypertensive treatment – no vs. yes) were related to waist circumference (dichotomized: <94 vs. ≥ 94 cm for men, <80 vs. ≥ 80 for women). All values for these variables are presented with the higher value as the second, e.g. male gender = 1 and female = 2, and “No” = 0 and “Yes” = 1.

†The ORs are arranged in descending order. The OR below 1.00 is inverted before sorting.

Table 4. Odds ratios (OR) after a logistic regression analysis (stepwise forward) for eight independent variables related to increased *fasting glucose*.

Independent variable*	b	SE	OR†	95% CI	
				Lower	Upper
Fasting insulin	1.16	0.079	3.19	2.74	3.73
Group	0.88	0.136	2.41	1.84	3.14
Gender	-0.80	0.079	0.45	0.38	0.52
Waist circumference	0.71	0.083	2.03	1.73	2.39
Blood pressure	0.54	0.095	1.72	1.43	2.07
Age	0.43	0.082	1.54	1.31	1.81
FHDf	0.38	0.077	1.46	1.26	1.70
Tobacco use	0.29	0.083	1.34	1.14	1.57
Constant	-4.41	0.233			

CI = Confidence Interval

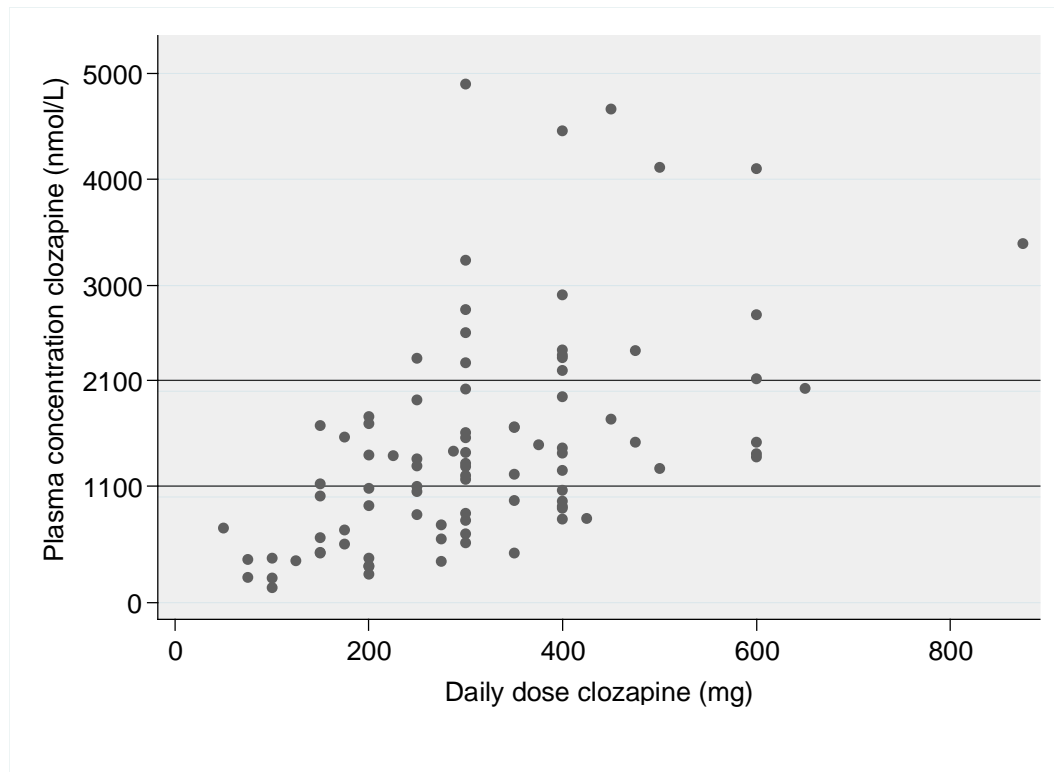
*Group (control vs. patient); gender (male vs. female); age (≤ 55 vs. > 55 yrs); tobacco use (smoking or snuffing – no vs. yes); first-degree relative with diabetes (FHD – no vs. yes); fasting insulin (< 114 pmol/L vs. ≥ 114 pmol/L); blood pressure (systolic ≥ 130 mmHg or diastolic ≥ 85 ; and mmHg or antihypertensive treatment – no vs. yes); and waist circumference (dichotomized: < 94 vs. ≥ 94 cm for men, < 80 vs. ≥ 80 for women) were related to fasting glucose (≥ 5.6 mmol/L – no vs. yes). All values for these variables are presented with the higher value as the second, e.g. male gender = 1 and female = 2, and “No” = 0 and “Yes” = 1.

†The ORs are arranged in descending order. ORs below 1.00 are first inverted.

4.2 PAPER II

There was a wide variation in plasma concentration of clozapine in the sample (mean = 1,615 nM, SD = 1,354 nM). Of 98 patients, 36 (37%) had plasma concentrations of clozapine within suggested therapeutic range (1,100-2,100 nM). Patients on a specific dose such as 300 mg/d had plasma concentrations within, as well as above and below the suggested range.

Figure 1. Relation between clozapine plasma level and daily dose. Measured clozapine concentrations at 12 hours after most recent dose plotted against daily dose in 92 patients* who had continuous treatment.



*Daily doses of clozapine were available for 92 patients. Two outliers, 7,136 and 8,070 nM, are not plotted in the graph.

Smoking was associated with lower plasma concentrations of clozapine ($P \leq .03$). We found no association between the clinical variables age, gender or weight and plasma concentration of clozapine.

The AA genotype in rs762551 (*CYP1A2*F*) was significantly associated to lower plasma concentrations of clozapine ($\beta = -2.0$; 95% CI -3.54 - 0.55). There was also a significant association in rs2032582 (*MDR1*), with GG genotype affecting plasma concentration of clozapine in the same direction ($\beta = -1.7$, 95% CI -3.45 - -0.05). The variance of clozapine concentration could only to a limited extent be explained by the two genetic variants and smoking status (R^2 linear = 0.162).

Table 5. Relation between independent variables and dose-corrected plasma clozapine concentration*

				95% Confidence Interval	
R^2 linear = 0.16					
Independent Variable [†]	β	Standard Error	$P \leq$	Lower	Upper
<i>CYP1A2</i> rs762551 AA genotype	-2.0	0.75	.008	-3.54	-0.55
Currently smoking	-1.8	0.76	.03	-3.31	-0.28
<i>MDR1</i> rs2032582 GG genotype	-1.7	0.85	.05	-3.45	-0.05
Constant	11.4	1.69	.001	8.02	14.72

*Multiple regression analysis (stepwise forward).

[†]rs762551 AA genotype (ref = no); smoking status (ref = no); rs2032582 GG genotype (ref = no)

We found no relation between plasma concentrations of clozapine and increased waist circumference or fasting glucose. However, the AA genotype in rs762551 (*CYP1A2**F), associated to lower plasma concentrations of clozapine was also associated to a lower risk of increased fasting glucose (OR = 0.27; 95% CI 0.10 - 0.72). Increased fasting glucose was also more common in males (OR = 5.28; 95% CI 1.88 – 14.89) and in older patients (OR = 1.08; 95% CI 1.03 - 1.13). There were no significant effects of the different genotypes, gender, age or smoking status on the risk of increased waist circumference.

Table 6. Relation between independent variables related to raised fasting plasma glucose (>5.6 mmol/L or antidiabetic treatment)*

Independent Variable [†]	Odds Ratio	Standard Error	<i>P</i> ≤	95% Confidence Interval	
				Lower	Upper
Sex	5.28	2.79	.002	1.88	14.89
rs762551 AA genotype	0.27	0.13	.009	0.10	0.72
Age (y)	1.08	0.03	.001	1.03	1.13

*Logistic regression analysis (stepwise forward).

[†]Sex (women = 0; men = 1); rs762551 AA genotype (no = 0; yes = 1).

4.3 PAPER III

Patients without antidiabetic pharmacotherapy had significantly higher fasting glucose as compared to control subjects, controlling for differences in age and gender (*P* = .001). There were significant differences in frequencies of diabetes and prediabetes (fasting glucose level 6.1 – 6.9 mM) between cases and control subjects, also controlling for differences in age and gender (*P* = .001; *P* = .001). Patients with schizophrenia were found to have significantly higher mean fasting glucose levels compared to patients with other psychiatric diagnoses. Both prediabetes and diabetes were approximately three times more frequent among patients with psychosis as compared to controls. The differences between patients with psychosis and control subjects in prevalence of prediabetes and fasting glucose 5.6-6.0 mM were most pronounced in younger age categories. Of patients with psychosis and prediabetes 77 percent also fulfilled the criteria for metabolic syndrome. There were no significant differences in antidiabetic pharmacological treatment comparing patients with psychosis and control subjects (*P* = .114).

The logistic regression analysis, investigating factors potentially associated with antidiabetic pharmacological treatment in patients with psychosis diagnosed with diabetes, yielded odds ratios as a measure of the association. In this analysis, lipid lowering treatment (OR = 6.74; 95% CI 1.88 – 24.13) and high fasting glucose (OR = 1.24; 95% CI 1.01 - 0.72) were associated with antidiabetic pharmacological treatment. Smoking status, metabolic syndrome, waist circumference, Global Assessment of Functioning scale, Clinical Global Impression scale, and antihypertensive treatment, duration of antipsychotic medication and number of months in hospital during the previous year did not show any association with antidiabetic pharmacological treatment. When patients with schizophrenia were analyzed separately a negative association, in addition to the two previous associations, was found between

duration of antipsychotic treatment and antidiabetic pharmacological treatment (OR = 0.88; 95 CI 0.79 - 0.97).

Table 7. Antidiabetic treatment, achievement of fasting glucose < 7 mmol/L in patients with psychotic disorders and control subjects with diabetes*

Glucose Levels	Psychosis Patients [†]	Control Subjects	<i>P</i>
DM diagnosis	94 (10.0) [‡]	69 (1.8)	.001 [§]
DM diagnosis and antidiabetic treatment	40 (42.6)	38 (55.1)	.114
Metabolic syndrome	33 (82.5)	ND	-
Fasting glucose < 7.0 mmol/L	13 (32.5)	12 (31.6)	.931
DM diagnosis and no antidiabetic treatment	54 (57.4)	31 (44.9)	.114
Metabolic syndrome	41 (77.4)	ND	-
Fasting glucose < 7.0 mmol/L	32 (59.3)	18 (58.1)	.914

*Data reported as number (%). Among controls only subjects who developed diabetes during the follow up period are included.

Abbreviations: DM, diabetes mellitus; ND, not determined.

[†]No. of patients varied due to sporadic missing information.

[‡]2 psychosis patients with insulin treatment without DM diagnosis were included.

[§]*P* from logistic regression models controlling for differences in sex and age.

Table 8. Increased fasting glucose in patients and control subjects without diabetes*

Glucose Levels	Psychosis Patients [†]	Control Subjects	<i>P</i>
No DM diagnosis	854 (90.1)	3839 (98.2)	.001 [‡]
Fasting glucose ≥ 7.0 mmol/L	43 (5.0)	41 (1.1)	.001 [‡]
Metabolic syndrome	32 (76.2)	ND	-
Fasting glucose 6.1-6.9 mmol/L	87 (10.2)	149 (3.8)	.001 [‡]
Metabolic syndrome	64 (77.1)	ND	-
Fasting glucose 5.6-6.0 mmol/L	160 (18.7)	355 (9.1)	.001 [‡]
Metabolic syndrome	103 (65.2)	ND	-

*Data reported as number (%).

Abbreviations: DM, diabetes mellitus; ND, not determined.

[†]No. of patients varied due to sporadic missing information.

[‡]*P* from logistic regression models controlling for differences in sex and age.

4.4 PAPER IV

Levels of biomarkers, in previous studies associated with elevated cardiovascular risk, were elevated above reference values in the current sample of patients with psychosis in regular treatment. A large proportion of patients, 29 percent (95% CI 23.9 - 34.1), had hsCRP levels of 3 – 10 mg/L, eight percent (95% CI 4.7-10.7) had pathological hsTnT levels and seven percent (95% CI 4.4-10.3) had pathological NT-BNP levels. Of patients with of hsTnT ≥ 15 ng/L, 44 percent had a diabetes diagnosis, 47 percent a diagnosis of hyperlipidemia, 28 percent a history of chest pain and only eleven percent were present smokers.

In the univariate analyses hsTnT and hsCRP were significantly correlated to more clinical cardiovascular risk factors than NT-BNP. HsTnT showed significant positive correlations to fasting glucose level, waist circumference, systolic blood pressure, diabetes diagnosis and diagnosis of hyperlipidemia. HsCRP had significant positive correlations to body mass index, triglyceride level, fasting glucose level, systolic and diastolic blood pressure HsCRP also showed a significant negative correlation to European quality of life-5 dimensions index (EQ-5D). NT-BNP had a significant positive correlation to previous diagnosis of acute myocardial infarction (AMI), and was negatively correlated to EQ-5D. The biomarkers were poorly correlated to each other with only a small but significant positive correlation between NT-

BNP and hsTnT. The regression model with hsTnT as the dependent variable and clinical cardiovascular risk factors as independent variables explained 37.9 percent of the variance of hsTnT (R^2 linear = 0.379). In this model, waist circumference was significantly associated with increased levels of hsTnT (standardized coefficient = 0.20; SE 0.02), controlling for differences in age, sex, plasma creatinine and fasting glucose level. Fasting glucose was also associated to increased levels of hsTnT (standardized coefficient = 0.21; SE 0.18), controlling for the same factors with the addition of waist circumference. Gender, age and plasma creatinine were also significantly associated with levels of hsTnT. None of the other independent cardiovascular risk factors entered in the model showed any significant association (Table 10).

Table 9. Clinical and demographic characteristics, hsTnT, NT-BNP and hsCRP in patients with psychosis

	Patients with psychosis	
	Men (n = 147)	Women (n = 153)
Age (years)	54 ± 7	58 ± 9**
Present smoker [†]	38 (45)	35 (43)
BMI (kg/m ²)	28 ± 5	29 ± 6
Waist (cm)	105 ± 14	99 ± 14*
Systolic blood pressure (mm Hg)	132 ± 18	131 ± 18
Diastolic blood pressure (mm Hg)	83 ± 11	84 ± 11
Fasting glucose (mmol/L)	6.0 ± 2.1	5.6 ± 1.1
Total Cholesterol (mmol/L)	5.3 ± 1.4	5.7 ± 1.1
LDL Cholesterol (mmol/L)	3.4 ± 0.9	3.6 ± 1.1
HDL Cholesterol (mmol/L)	1.1 ± 0.3	1.4 ± 0.4***
Triglycerides (mmol/L)	1.8 ± 1.6	1.4 ± 0.7*
Biomarkers		
HsTnT (ng/L)	7 ± 6	4 ± 5***
HsTnT ≥ 15 ng/L	10 (15)	5 (8)
NT-BNP (ng/L)	86 ± 442	95 ± 122***
NT-BNP > 100 ng/L	11 (16)	27 (42)***
NT-BNP > 150 ng/L	4 (6)	14 (22)*
HsCRP (mg/L)	3.2 ± 3.7	3.6 ± 5.2
HsCRP < 1	35 (51)	29 (45)
HsCRP 1-3	33 (49)	31 (48)
HsCRP > 3	32 (47)	39 (60)
HsCRP 3-10	26 (38)	32 (49)
HsCRP > 10	6 (9)	7 (11)

Data reported as mean ± standard deviation or % (number).

* p < 0.01, ** p<0.001, *** p<0.0001

[†]No. of patients varied due to sporadic missing information.

Abbreviations: BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; BNP, N-Terminal Pro-B-Type Natriuretic Peptide; CRP, high-sensitive C-reactive protein

Table 10. Relation between independent variables and hs-TnT in a multiple regression analysis (stepwise forward).

Independent Variable	Unstandardized coefficients		t	Standardized coefficients
	B	SE		Beta
Age	0.31	0.04	7.78	0.43
Gender (1= man; 2= woman)	-2.35	0.74	-3.19	-0.20
Waist circumference	0.08	0.02	3.58	0.20
Plasma creatinine	0.07	0.02	3.20	0.19
Fasting glucose	0.71	0.18	3.91	0.21
Constant	-25.90	3.75	-6.91	-
R ² linear	0.379			

Abbreviations: SE= Standard Error

5 DISCUSSION

5.1 STUDY I

In our opinion, the most important finding was the substantially increased risk in patients with psychosis of having increased waist circumference and increased fasting glucose compared to control subjects. The elevated risk of increased waist circumference was significant, controlling for differences in fasting insulin, sex, blood pressure, fasting glucose, family history of diabetes, age and tobacco use. The increased risk of higher fasting glucose was observed controlling for the same factors with addition of waist circumference. This implies that the mechanism by which antipsychotic drugs can raise fasting glucose levels and cause glucose disturbances and diabetes is not only through weight gain and increased insulin resistance. Increased hepatic glucose output (HGO) and direct effects on insulin and glucagon secretion from pancreatic islets have been suggested as other important mechanisms (Smith et al., 2014).

Smoking was significantly more common among patients with psychosis compared to controls. A selection bias with “super healthy” control subjects might be present and overestimate differences. In contrast, the cases are outpatients, and compared to an inpatient sample, they are likely to have lower smoking prevalence. This is supported by the fact that the prevalence of smoking in the current material was lower than the prevalence reported in a large retrospective cohort of patients with severe mental illness (44 vs 61%) (Osborn et al., 2006). Furthermore, the sample in the current study included all patients in regular psychosis outpatient treatment. Smoking prevalence is likely to be lower in such a sample compared to smoking prevalence in a sample consisting of schizophrenia patients only. In conclusion, smoking still seems to be considerably more prevalent in patients with psychosis compared to controls, possibly contributing to the excess cardiovascular mortality and morbidity.

5.2 STUDY II

There was a wide variation in plasma concentration of clozapine with 37 percent of patients within the suggested therapeutic range (1,100 – 2,100 nM). Patients on a specific dose of clozapine such as 300 mg/d had plasma concentrations within, above and below therapeutic range. Individual clinical and genetic factors could only to a limited extent explain the variance in concentrations of clozapine. The factors that seemed to affect clozapine concentration were smoking habits, variants of *CYP1A2* and variants of *MDR1*. In line with results from study I, increased fasting glucose was associated with plasma concentrations of clozapine, independent of increased waist circumference. The results also indirectly suggest that risk of increased fasting glucose is concentration dependent. One possible explanation for the absence of association between metabolism affecting factors, plasma concentration of clozapine and waist circumference might be that it is weight increase and the subsequent increase in waist circumference that is associated to plasma concentration and not current

waist circumference. Furthermore, the results confirm that CYP1A2 is central in the metabolism of clozapine.

5.3 STUDY III

The main finding of the study was the high frequency of prediabetes (FPG 6.1 – 6.9 mM) in patients with psychosis compared to controls (10.2 vs 3.8%). In patients with psychosis and prediabetes, 77 percent also fulfilled the criteria for metabolic syndrome. Patients with metabolic syndrome have a high risk of developing manifest diabetes. There are established life-style interventions and pharmacotherapies to prevent or delay the onset of diabetes (Diabetes Prevention Program Research et al., 2009; Merlotti, Morabito, & Pontiroli, 2014). The present study proposes that reduced progression from prediabetes to diabetes should be a treatment goal for patients with psychosis. Considering previous progression rates for obese subjects of 10-12 percent (Knowler et al., 2002; Tuomilehto et al., 2001) to be valid also in patients with psychosis, approximately eight to ten patients out of 854 progress to manifest diabetes yearly. Regular use of Metformin would be expected to lower the progression rate substantially and has previously been reported to lower it by as much as 30% (Knowler et al., 2002). Patients with psychosis and no diagnosis of diabetes had higher fasting plasma glucose levels compared to controls. Furthermore, more patients with psychosis and no diabetes diagnosis had $\text{FPG} \geq 7.0 \text{ mM}$ compared to controls. This could be explained by patients having a longer time to diabetes diagnosis compared to controls. However, this conclusion could be afflicted by bias since controls with diabetes at baseline were excluded.

5.4 STUDY IV

The three biomarkers, NT-BNP, hsTnT and hsCRP were elevated in blood samples from psychosis patients without pre-existing cardiovascular disease during regular anti-psychotic treatment. A large proportion 29 percent (95% CI 23.9 - 34.1) had hsCRP levels of 3 – 10 mg/L. Eight percent (95% CI 4.8-10.6) had pathological hsTnT and seven percent (95% CI 4.4-10.3) pathological NT-BNP levels. Furthermore, one percent had pathological values in all three biomarkers. With increased levels of biomarkers, it was most common that a single biomarker was clearly dominating, i.e. was above reference value. Together with the fact that NT-BNP, hsTnT and hsCRP reflect wall stress, myocardial cell damage and inflammation respectively this could imply that each biomarker contribute with unique information. This is also supported by NT-BNP, hsTnT and hsCRP being poorly correlated to each other.

HsCRP showed significant positive correlations to different measures of overweight in the univariate analyses, in line with previous findings suggesting an association between inflammation and metabolic disturbances in these patients (Miller, Mellor, & Buckley, 2013). Other processes engaging the immune system such as chronic obstructive lung disease and the psychotic disease itself can possibly also contribute to the elevated levels of hsCRP in

these patients. The majority of patients with chronic inflammatory conditions are however excluded when patients with CRP levels of less than 10 mg/L are studied. Patients with CRP levels of 3 – 10 mg/L are also the group of patients previously associated to the highest risk of overweight (Wium-Andersen, Orsted, Nielsen, & Nordestgaard, 2013).

Clinical cardiovascular risk factors were more closely associated to hsTnT as compared to hsCRP and NT-BNP. HsTnT, in contrast to NT-BNP and hsCRP, was significantly associated with larger waist circumference and higher fasting glucose level in a regression analysis with established clinical risk factors as independent variables. Unfortunately, the study was not able to study potential advantage of biomarkers of increased cardiovascular risk over traditional clinical risk factors e.g. diabetes, tobacco use, blood pressure, body mass index, and waist circumference. It is important to point out that the best biomarker of increased cardiovascular risk is not the biomarker most closely associated to clinical already established risk factors but a biomarker which identifies high-risk individuals not identified by traditional risk factors. If biomarkers do add information for better stratification of cardiovascular risk, they should be used as guidance for counteraction and reduction of cardiovascular risk among patients with psychosis.

6 CONCLUSIONS

There is a demand for biomarkers for somatic health monitoring and as guidance for prevention of cardiovascular events in patients with psychotic disorder. Psychiatrists should collaborate closely with general practitioners and cardiologists. Basic pharmacological treatment of medical conditions, monitoring of risk factors and organizational structures of somatic referral should be handled by the psychiatrist.

Psychosis is associated to higher fasting glucose levels and increased waist circumference compared to controls. Furthermore, the frequency of smoking is clearly higher among patients with psychosis compared to controls. Also, we conclude that the mechanisms by which antipsychotic agents cause glycemic disturbances are complex and as yet far from fully understood.

Regular therapeutic drug monitoring is advised for patients on antipsychotic treatment with clozapine. The main reasons for this are the following: (1) it is difficult to predict plasma concentration of clozapine even when clinical and genetic factors are considered; (2) only 37 percent of patients studied had plasma concentrations within suggested therapeutic interval; (3) on a specified dose patients had plasma concentrations within, above and below suggested therapeutic interval; (4) there were patients with exceptionally high concentrations. CYP1A2 is important in the metabolism of clozapine and functional gene variants are common. Of the clinical factors investigated, smoking had the largest effect on plasma concentrations of clozapine. For clozapine, a strong case could also be made in favor of systematic prospective treatment studies to improve the assessment of recommended drug levels. In addition, a strong case could also be made for monitoring other antipsychotic drugs, which may increase treatment effects and reduce drug adverse effects.

Approximately one out of ten patients with psychosis had prediabetes (FPG 6.1 – 6.9 mM). Prediabetes is more common in patients with psychosis compared to population controls. A majority of patients with psychosis and prediabetes also fulfill the criteria for metabolic syndrome. This suggests that the progression rate from prediabetes to manifest diabetes is higher among patients with psychosis compared to the population. Treatment of prediabetes in patients with psychosis with life-style interventions and/or pharmacotherapy is proposed in this thesis, and reduced progression to diabetes as an important treatment goal. Biomarkers associated with cardiovascular risk in previous studies were elevated in patients with psychosis. Since endpoint data of cardiovascular events were not available, it was not possible to assess if biomarkers add information to risk stratification.

7 IMPLICATIONS FOR FUTURE RESEARCH

Future studies should focus on assessing modifiable cardiovascular risk factors among patients with psychosis, and testing the effect sizes in prospective randomized clinical efficacy studies. Reduced progression of prediabetes to diabetes, weight reduction and smoking cessation should be given extra attention. Concerning prediabetes, prospective studies should evaluate the potential preventive effect on diabetes of life-style programmes and pharmacological interventions with metformin and possibly newer agents.

Future studies of clozapine should focus on collecting data enabling us to understand why patients on clozapine discontinue treatment. Broadening our understanding of the effect of clozapine is a critical part to the solution of this dilemma. We are planning a clozapine project to shed some light on these issues. In this project we aim to collect data on effect of treatment and enroll patients currently on clozapine treatment and patients who have recently discontinued treatment. A special unit within the organization specifically for patients on clozapine treatment, in conformity with patients on lithium treatment, is going to be the structure of the project. One interesting part of the project involves induced pluripotent stem cells (IPS). With this technique it is possible to grow the patient's own dopamine cells from fibroblasts attained with a skin biopsy. Since the antipsychotic effect of neuroleptic drugs is thought to be mediated by the dopamine system, specifically by blocking the dopamine D2 receptor, it is possible that the systems differ between responders and non-responders. With this technique we should be able to study dopamine cells and the dopamine D2 receptors expressed on dopamine cells. Clozapine is well known to have direct and indirect effects also on other receptors, including the serotonin receptor and the glutamate receptor. The complex effects of clozapine on different signaling systems is thought to be the explanation for the unique effect of the compound. From neuronal stem cells it is possible to cultivate cells expressing different receptors, including the glutamate receptor. In this project we also intend to study genes, gene expression, plasma levels of clozapine and cerebrospinal fluid.

Prospective studies should evaluate the potential advantage of biomarkers of cardiovascular risk in patients with psychosis in regular treatment. A confirmed advantage of biomarkers should lead to them being added to clinical guidelines for management of patients with severe mental illness. As a next step, our research team plans a follow-up study with the cohort from study IV as baseline.

A general implication of the findings of this thesis is that there is likely to be substantial clinical improvement for psychosis patients by further clinical studies along the directions of the studies included.

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9 REFERENCES

- Aas, I. H. (2011). Guidelines for rating Global Assessment of Functioning (GAF). *Ann Gen Psychiatry*, 10, 2. doi: 10.1186/1744-859X-10-2
- Ahlers, E., Hahn, E., Ta, T. M., Goudarzi, E., Dettling, M., & Neuhaus, A. H. (2014). Smoking improves divided attention in schizophrenia. *Psychopharmacology (Berl)*, 231(19), 3871-3877. doi: 10.1007/s00213-014-3525-2
- Alawami, M., Wasywich, C., Cicovic, A., & Kenedi, C. (2014). A systematic review of clozapine induced cardiomyopathy. *International Journal of Cardiology*, 176(2), 315-320. doi: 10.1016/j.ijcard.2014.07.103
- Alberti, K. G., Zimmet, P., & Shaw, J. (2006). Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med*, 23(5), 469-480. doi: 10.1111/j.1464-5491.2006.01858.x
- Alberti, K. G., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*, 15(7), 539-553. doi: 10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S
- American Psychiatric Association. (2000). *Diagnostic criteria from DSM-IV-TR*. Washington, D.C.: American Psychiatric Association.
- Ascher-Svanum, H., Zhu, B., Faries, D., Landbloom, R., Swartz, M., & Swanson, J. (2006). Time to discontinuation of atypical versus typical antipsychotics in the naturalistic treatment of schizophrenia. *BMC Psychiatry*, 6, 8. doi: 10.1186/1471-244x-6-8
- Bersani, F. S., Capra, E., Minichino, A., Pannese, R., Girardi, N., Marini, I., . . . Biondi, M. (2011). Factors affecting interindividual differences in clozapine response: a review and case report. *Hum Psychopharmacol*, 26(3), 177-187. doi: 10.1002/hup.1191
- Brinkmann, U. (2002). Functional polymorphisms of the human multidrug resistance (MDR1) gene: correlation with P glycoprotein expression and activity in vivo. *Novartis Found Symp*, 243, 207-210; discussion 210-202, 231-205.
- Brown, S. (1997). Excess mortality of schizophrenia. A meta-analysis. *Br J Psychiatry*, 171, 502-508.
- Busner, J., & Targum, S. D. (2007). The clinical global impressions scale: applying a research tool in clinical practice. *Psychiatry (Edgmont)*, 4(7), 28-37.
- Chan, J. M., Rimm, E. B., Colditz, G. A., Stampfer, M. J., & Willett, W. C. (1994). Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care*, 17(9), 961-969.
- Colditz, G. A., Willett, W. C., Rotnitzky, A., & Manson, J. E. (1995). Weight gain as a risk factor for clinical diabetes mellitus in women. *Ann Intern Med*, 122(7), 481-486.
- Consoli, G., Lastella, M., Ciapparelli, A., Catena Dell'Osso, M., Ciofi, L., Guidotti, E., . . . Di Paolo, A. (2009). ABCB1 polymorphisms are associated with clozapine plasma levels in psychotic patients. *Pharmacogenomics*, 10(8), 1267-1276. doi: 10.2217/pgs.09.51
- Coulter, D. M., Bate, A., Meyboom, R. H., Lindquist, M., & Edwards, I. R. (2001). Antipsychotic drugs and heart muscle disorder in international pharmacovigilance: data mining study. *BMJ*, 322(7296), 1207-1209.

- Crilly, J. (2007). The history of clozapine and its emergence in the US market: a review and analysis. *Hist Psychiatry*, 18(1), 39-60.
- de Koning, L., Merchant, A. T., Pogue, J., & Anand, S. S. (2007). Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. *Eur Heart J*, 28(7), 850-856. doi: 10.1093/eurheartj/ehm026
- Despres, J. P., Lemieux, I., & Prud'homme, D. (2001). Treatment of obesity: need to focus on high risk abdominally obese patients. *BMJ*, 322(7288), 716-720.
- Diabetes Prevention Program Research, Group, Knowler, W. C., Fowler, S. E., Hamman, R. F., Christophi, C. A., Hoffman, H. J., . . . Nathan, D. M. (2009). 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. *Lancet*, 374(9702), 1677-1686. doi: 10.1016/S0140-6736(09)61457-4
- Eckel, R. H., Alberti, K. G., Grundy, S. M., & Zimmet, P. Z. (2010). The metabolic syndrome. *Lancet*, 375(9710), 181-183. doi: 10.1016/S0140-6736(09)61794-3
- Elbe, D., & Savage, R. (2010). How does this happen? Part I: mechanisms of adverse drug reactions associated with psychotropic medications. *J Can Acad Child Adolesc Psychiatry*, 19(1), 40-45.
- Emery, E. M., Schmid, T. L., Kahn, H. S., & Filozof, P. P. (1993). A review of the association between abdominal fat distribution, health outcome measures, and modifiable risk factors. *Am J Health Promot*, 7(5), 342-353.
- Erickson-Ridout, K. K., Sun, D., & Lazarus, P. (2012). Glucuronidation of the second-generation antipsychotic clozapine and its active metabolite N-desmethyloclozapine. Potential importance of the UGT1A1 A(TA)(7)TAA and UGT1A4 L48V polymorphisms. *Pharmacogenet Genomics*, 22(8), 561-576. doi: 10.1097/FPC.0b013e328354026b
- Eriksson, A. K., Ekblom, A., Granath, F., Hilding, A., Efendic, S., & Ostenson, C. G. (2008). Psychological distress and risk of pre-diabetes and Type 2 diabetes in a prospective study of Swedish middle-aged men and women. *Diabet Med*, 25(7), 834-842. doi: 10.1111/j.1464-5491.2008.02463.x
- Faber, M. S., Jetter, A., & Fuhr, U. (2005). Assessment of CYP1A2 activity in clinical practice: why, how, and when? *Basic Clin Pharmacol Toxicol*, 97(3), 125-134. doi: 10.1111/j.1742-7843.2005.pto_973160.x
- Goff, D. C., Sullivan, L. M., McEvoy, J. P., Meyer, J. M., Nasrallah, H. A., Daumit, G. L., . . . Lieberman, J. A. (2005). A comparison of ten-year cardiac risk estimates in schizophrenia patients from the CATIE study and matched controls. *Schizophr Res*, 80(1), 45-53. doi: 10.1016/j.schres.2005.08.010
- Gothefors, D., Adolfsson, R., Attvall, S., Erlinge, D., Jarbin, H., Lindstrom, K., . . . Swedish Psychiatric Association. (2010). Swedish clinical guidelines--prevention and management of metabolic risk in patients with severe psychiatric disorders. *Nord J Psychiatry*, 64(5), 294-302. doi: 10.3109/08039488.2010.500397
- Gurillo, P., Jauhar, S., Murray, R. M., & MacCabe, J. H. (2015). Does tobacco use cause psychosis? Systematic review and meta-analysis. *Lancet Psychiatry*, 2(8), 718-725. doi: 10.1016/S2215-0366(15)00152-2
- Hennen, J., & Baldessarini, R. J. (2005). Suicidal risk during treatment with clozapine: a meta-analysis. *Schizophr Res*, 73(2-3), 139-145. doi: 10.1016/j.schres.2004.05.015

- Hert, M., Detraux, J., van Winkel, R., Yu, W. P., & Correll, C. U. (2012). Metabolic and cardiovascular adverse effects associated with antipsychotic drugs. *Nature Reviews Endocrinology*, 8(2), 114-126. doi: 10.1038/nrendo.2011.156
- Kane, J., Honigfeld, G., Singer, J., & Meltzer, H. (1988). Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Arch Gen Psychiatry*, 45(9), 789-796.
- Kane, J. M. (1996). Treatment-resistant schizophrenic patients. *J Clin Psychiatry*, 57 Suppl 9, 35-40.
- Khasawneh, F. T., & Shankar, G. S. (2014). Minimizing cardiovascular adverse effects of atypical antipsychotic drugs in patients with schizophrenia. *Cardiol Res Pract*, 2014, 273060. doi: 10.1155/2014/273060
- Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., . . . Diabetes Prevention Program Research, Group. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*, 346(6), 393-403. doi: 10.1056/NEJMoa012512
- Larsson, H., Lindgarde, F., Berglund, G., & Ahren, B. (2000). Prediction of diabetes using ADA or WHO criteria in post-menopausal women: a 10-year follow-up study. *Diabetologia*, 43(10), 1224-1228. doi: 10.1007/s001250051516
- Laursen, T. M., Wahlbeck, K., Hallgren, J., Westman, J., Osby, U., Alinaghizadeh, H., . . . Nordentoft, M. (2013). Life expectancy and death by diseases of the circulatory system in patients with bipolar disorder or schizophrenia in the Nordic countries. *PLoS One*, 8(6), e67133. doi: 10.1371/journal.pone.0067133
- Leucht, S., Cipriani, A., Spineli, L., Mavridis, D., Orey, D., Richter, F., . . . Davis, J. M. (2013). Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet*, 382(9896), 951-962. doi: 10.1016/S0140-6736(13)60733-3
- Mackin, P., Bishop, D., Watkinson, H., Gallagher, P., & Ferrier, I. N. (2007). Metabolic disease and cardiovascular risk in people treated with antipsychotics in the community. *Br J Psychiatry*, 191, 23-29. doi: 10.1192/bjp.bp.106.031716
- Marder, S. R., Essock, S. M., Miller, A. L., Buchanan, R. W., Casey, D. E., Davis, J. M., . . . Shon, S. (2004). Physical health monitoring of patients with schizophrenia. *Am J Psychiatry*, 161(8), 1334-1349. doi: 10.1176/appi.ajp.161.8.1334
- Merlotti, C., Morabito, A., & Pontiroli, A. E. (2014). Prevention of type 2 diabetes; a systematic review and meta-analysis of different intervention strategies. *Diabetes Obes Metab*, 16(8), 719-727. doi: 10.1111/dom.12270
- Miller, B. J., Mellor, A., & Buckley, P. (2013). Total and differential white blood cell counts, high-sensitivity C-reactive protein, and the metabolic syndrome in non-affective psychoses. *Brain Behav Immun*, 31, 82-89. doi: 10.1016/j.bbi.2012.08.016
- Olsson, H., Lennestål, R., & Hagg, S. (2015). Inför ny regim för blodprovskontroller vid klopabinbehandling. *Lakartidningen*, 112.
- Osborn, D. P., Nazareth, I., & King, M. B. (2006). Risk for coronary heart disease in people with severe mental illness: cross-sectional comparative study in primary care. *Br J Psychiatry*, 188, 271-277. doi: 10.1192/bjp.bp.104.008060

- Osby, U., Correia, N., Brandt, L., Ekblom, A., & Sparen, P. (2000). Mortality and causes of death in schizophrenia in Stockholm county, Sweden. *Schizophrenia Research*, 45(1-2), 21-28.
- Palmer, B. A., Pankratz, V. S., & Bostwick, J. M. (2005). The lifetime risk of suicide in schizophrenia: a reexamination. *Arch Gen Psychiatry*, 62(3), 247-253. doi: 10.1001/archpsyc.62.3.247
- Saha, S., Chant, D., & McGrath, J. (2007). A systematic review of mortality in schizophrenia: is the differential mortality gap worsening over time? *Archives of General Psychiatry*, 64(10), 1123-1131. doi: 10.1001/archpsyc.64.10.1123
- Saha, S., Chant, D., Welham, J., & McGrath, J. (2005). A systematic review of the prevalence of schizophrenia. *PLoS Med*, 2(5), e141. doi: 10.1371/journal.pmed.0020141
- Schinnar, A. P., Rothbard, A. B., Kanter, R., & Jung, Y. S. (1990). An empirical literature review of definitions of severe and persistent mental illness. *Am J Psychiatry*, 147(12), 1602-1608.
- Segarra, R., Zabala, A., Eguiluz, J. I., Ojeda, N., Elizagarate, E., Sanchez, P., . . . Gutierrez, M. (2011). Cognitive performance and smoking in first-episode psychosis: the self-medication hypothesis. *Eur Arch Psychiatry Clin Neurosci*, 261(4), 241-250. doi: 10.1007/s00406-010-0146-6
- Sheweita, S. A. (2000). Drug-metabolizing enzymes: mechanisms and functions. *Curr Drug Metab*, 1(2), 107-132.
- Smith, G. C., Zhang, Z. Y., Mulvey, T., Petersen, N., Lach, S., Xiu, P., . . . Shepherd, P. R. (2014). Clozapine directly increases insulin and glucagon secretion from islets: implications for impairment of glucose tolerance. *Schizophr Res*, 157(1-3), 128-133. doi: 10.1016/j.schres.2014.05.003
- Strom, B. L., Eng, S. M., Faich, G., Reynolds, R. F., D'Agostino, R. B., Ruskin, J., & Kane, J. M. (2011). Comparative mortality associated with ziprasidone and olanzapine in real-world use among 18,154 patients with schizophrenia: The Ziprasidone Observational Study of Cardiac Outcomes (ZODIAC). *Am J Psychiatry*, 168(2), 193-201. doi: 10.1176/appi.ajp.2010.08040484
- Tuomilehto, J., Lindstrom, J., Eriksson, J. G., Valle, T. T., Hamalainen, H., Ilanne-Parikka, P., . . . Finnish Diabetes Prevention Study, Group. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med*, 344(18), 1343-1350. doi: 10.1056/NEJM200105033441801
- Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., . . . Vos, T. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet*, 382(9904), 1575-1586. doi: 10.1016/S0140-6736(13)61611-6
- Wium-Andersen, M. K., Orsted, D. D., Nielsen, S. F., & Nordestgaard, B. G. (2013). Elevated C-reactive protein levels, psychological distress, and depression in 73, 131 individuals. *JAMA Psychiatry*, 70(2), 176-184. doi: 10.1001/2013.jamapsychiatry.102